



Appl. 09/475,158; Filed: December 30, 1999
Dkt. No. 0609.4780001; Group Art Unit: 1646
Inventors: GARDELLA *et al.*; Tel.: (202) 371-2600
Title: PTH Functional Domain Conjugate Peptides,
Derivatives Thereof and Novel Tethered Ligand-Receptor
Molecules

PG5 PTH(1-9)/(Gly)₉PTH(15-31)

1
A-V-S-E-I-Q-L-M-H-g-g-g-g-L-N-S-M-E-R-V-E-W-L-R-K-K-L-Q-D-V-NH₂ (SEQ ID NO:9)

31

1 GCUGUUUCCG AAUCCAGCU GAUGCACGGU GGUGGUGGUG GUCUGAACUC

51 CAUGGAACGU GUUGAUGGC UGCGUAAAA ACUGCAGGAC GUU (SEQ ID NO:14)

PG7 PTH(1-9)/(Gly)₉PTH(17-31)

1
A-V-S-E-I-Q-L-M-H-g-g-g-g-g-S-M-E-R-V-E-W-L-R-K-K-L-Q-D-V-NH₂ (SEQ ID NO:11)

31

1 GCUGUUUCCG AAUCCAGCU GAUGCACGGU GGUGGUGGUG GUGGUGGUUC

51 CAUGGAACGU GUUGAUGGC UGCGUAAAA ACUGCAGGAC GUU (SEQ ID NO:15)

PG9 PTH(1-5)/(Gly)₉PTH(15-31)

1
A-V-S-E-I-g-g-g-g-g-g-g-L-N-S-M-E-R-V-E-W-L-R-K-K-L-Q-D-V-NH₂ (SEQ ID NO:13)

31

1 GCUGUUUCCG AAUCCGGUGG UGGUGGUGGU GGUGGUGGUG GUCUGAACUC

51 CAUGGAACGU GUUGAUGGC UGCGUAAAA ACUGCAGGAC GUU (SEQ ID NO:16)

FIG.1



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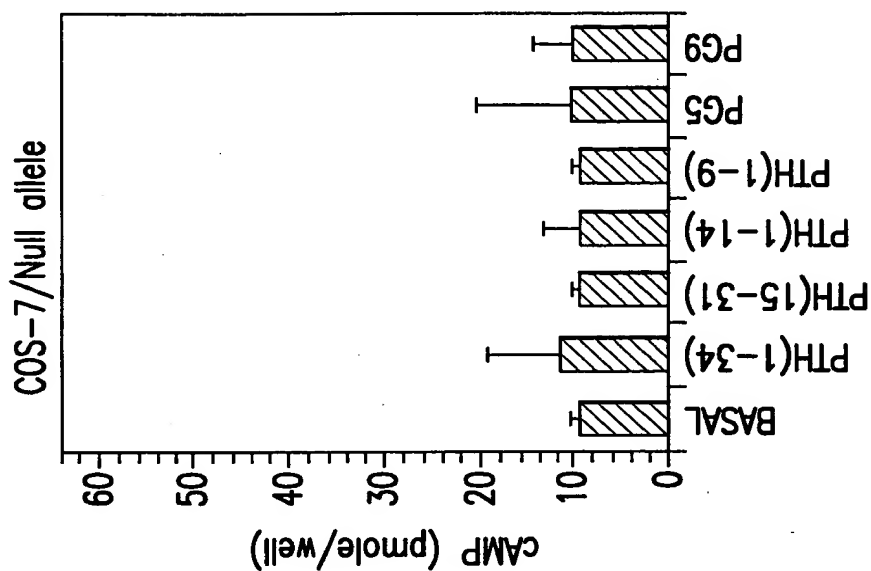
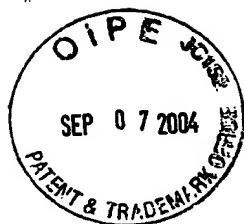
Family B Ligands

This is a list-- not an alignment:

	1	5	15	25	
hpth	1	SVSEI	QLMHNLGKHL	NSMERVEWLR	KKLQDVHNF (SEQ ID NO:17)
hpthrp	1	AVSEH	QLLHDKGKSI	QDLRRRFFFLH	HLIAEIHTA (SEQ ID NO:18)
hpacap	1	HSDGI	FTDSYSRYRK	QMAVKKYLA	VLGKRYKQR VNK (SEQ ID NO:19)
hvip	1	HSDAV	FTDNYTRLRK	QMAVKKYLS	ILN (SEQ ID NO:20)
hgrf	1	YADAI	FTNSYRKVLG	QLSARKLLQD	IMSR (SEQ ID NO:21)
hphm	1	HADGV	FTSDFSLLG	QLSAKKYLES	LM (SEQ ID NO:22)
hg1p1	7	HAEGT	FTSDVSSYLE	GQAAKEFIAM	LVKGRG (SEQ ID NO:23)
hglucagon	1	HSQGT	FTSDYSKYLD	SRRAQDFVQW	LMNT (SEQ ID NO:24)
gip	1	YAEGT	FISDYSIAMD	KIHQQDFVNW	LLAQKGKN DWKHNITQ (SEQ ID NO:25)
hsecretin	1	HSDGT	FTSELSRLRE	GARLQRLQ	LV (SEQ ID NO:26)
hcalcitonin	1	CGNLS	TCMLGTYTQD	FNKFHTFPQT	AIGVGAP (SEQ ID NO:27)
hcgrp-2	1	ACNTA	TCVTHRLAGL	LSRSGGMVKS	NFVPTNVGSKAF (SEQ ID NO:28)
hcgrp1	1	ACDTA	TCVTHRLAGL	LSRSGGVWKN	NFVPTNVGSKAF (SEQ ID NO:29)
hamylin	1	KCNTA	TCATQRLANF	LVHSSNNFGA	ILSSTNVGSNTY (SEQ ID NO:30)
hadrenomedu	1	GCRFG	TCTVQKLAHQ	IYQFTDKDKD	NVAPRSKISPP (SEQ ID NO:31)
hcrf	1	SEEP	ISLDLTFHLL	REVLEMARAE	QLAQQAHSNRKLMELI (SEQ ID NO:32)
sauvagine	1	EEPPI	SIDLSELLR	KMIEIEKQEK	EKQQAANNRLLLDTI (SEQ ID NO:33)
msdh	1	TGAQS	LSIVAPLDVL	RQRLMNEINR	RRMRELQGSRIQQNRQLLTSI (SEQ ID NO:34)
Maxadilan	1	CDATC	QFRKAIDDCQ	KQAHSNVLQ	TSVQTATFTSMDTSQPLPGNSVFKECMKQKKKEFSSGK (SEQ ID NO:35)

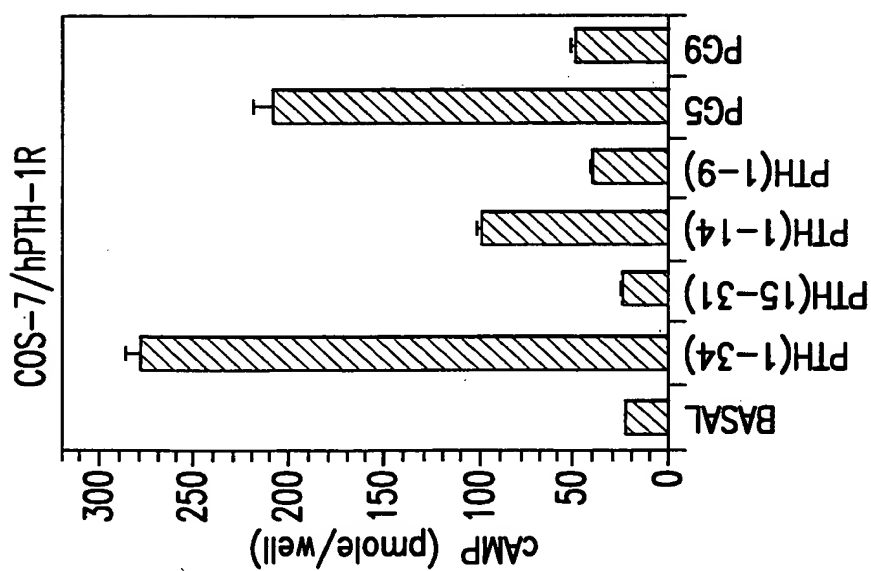
FIG.2

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PEPTIDES @ 10 μ M
 EXCEPT PTH(1-34)=1 μ M

FIG.3A-2

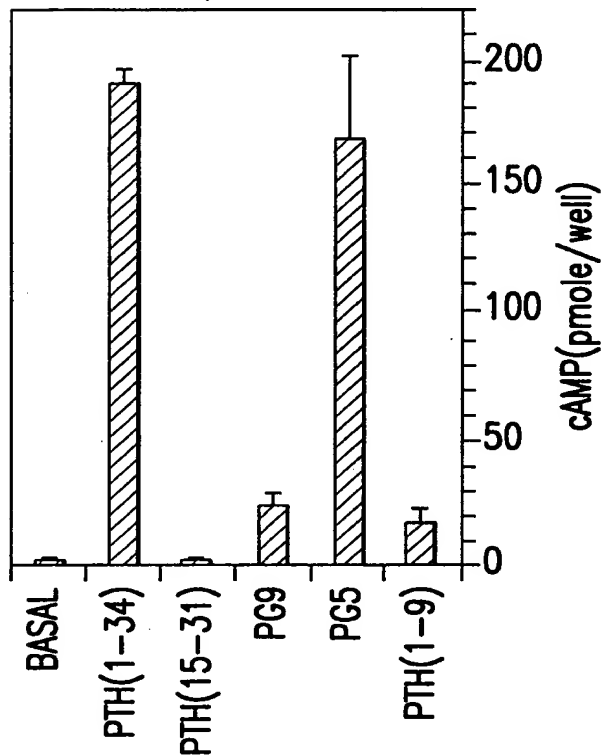


PEPTIDES @ 10 μ M
 EXCEPT PTH(1-34)=1 μ M

FIG.3A-1

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COS-7/hPTH-1 R



PEPTIDES @ 10 μ M
 EXCEPT PTH(1-34) AT 1 μ M

FIG.3B

COS-7/hPTH-1 RECEPTOR

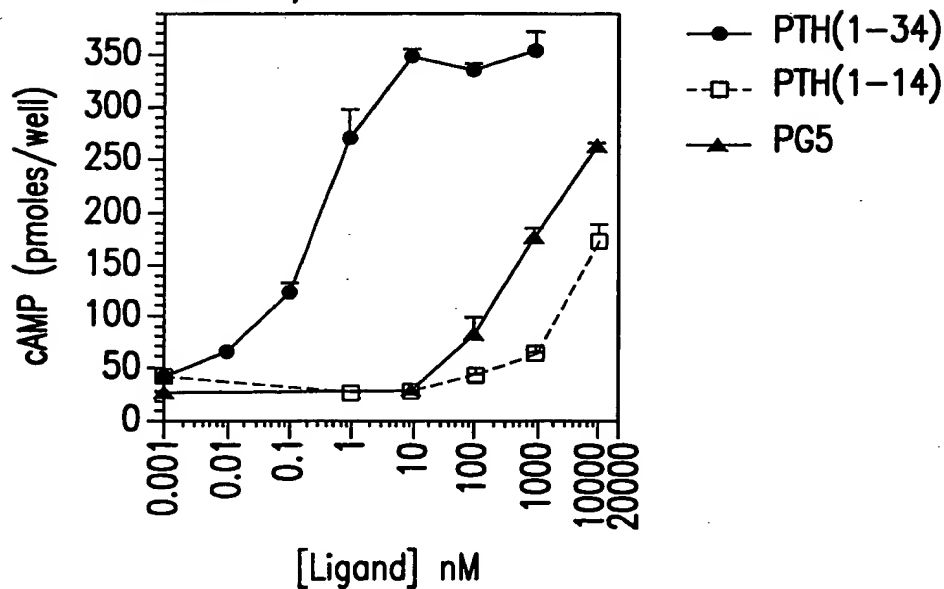


FIG.4A

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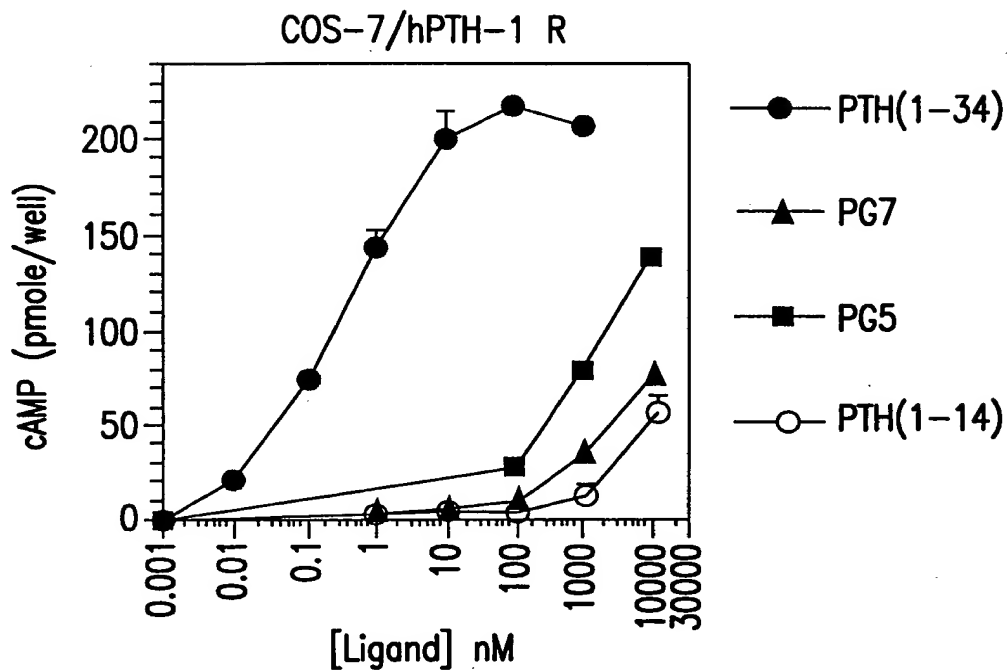


FIG. 4B

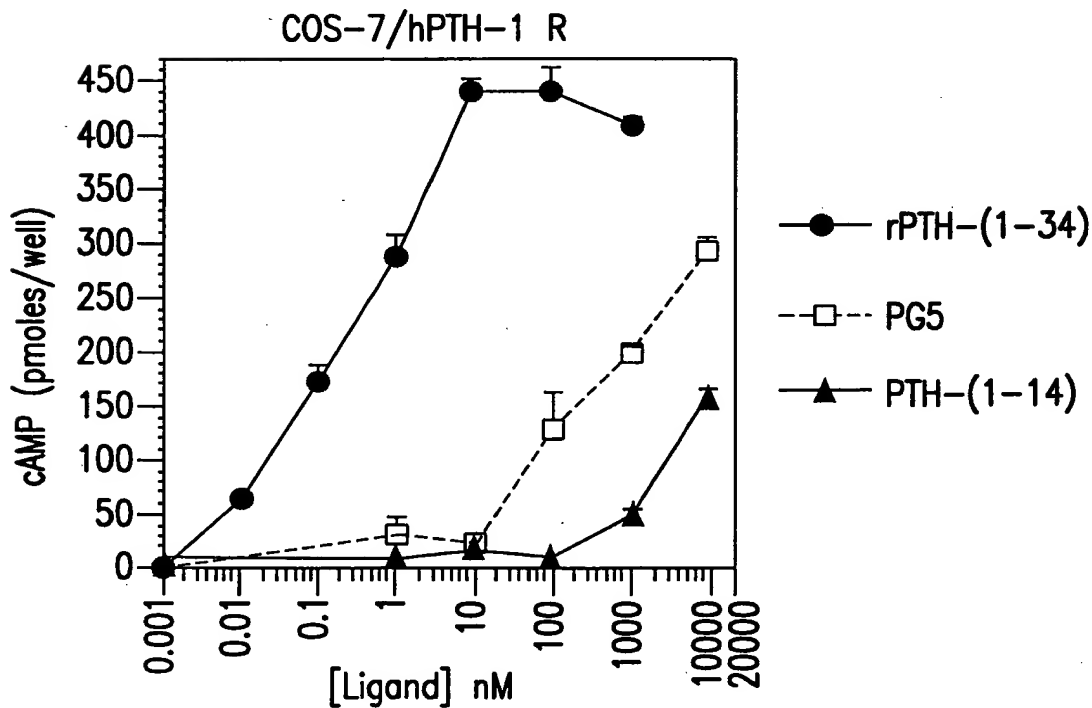
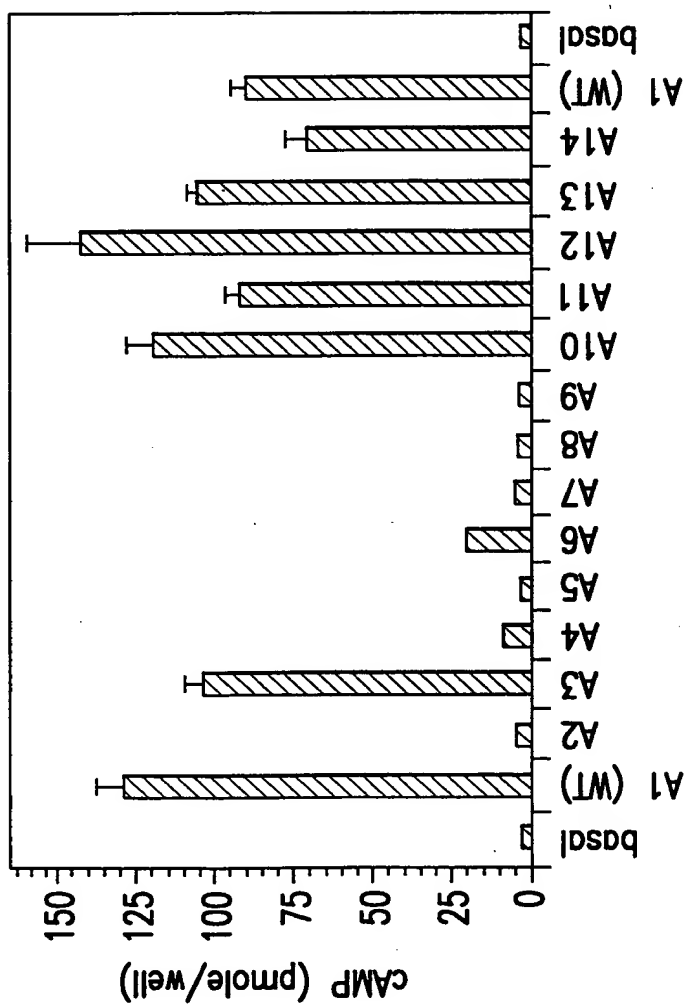


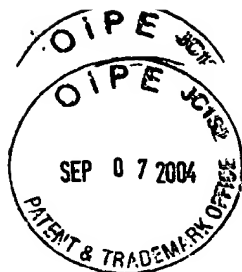
FIG. 4C

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A-V-S-E-I-Q-L-M-H-N-L-G-K-H

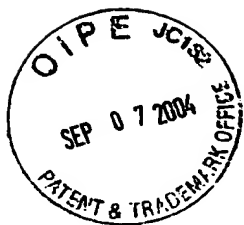
FIG.5



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ALANINE SCAN OF PTH (17-31)			
NATIVE PTH(17-31)		BINDING IC ₅₀ (μ M) 1.0 \pm 0.1	n 14
POSITION	SUBSTITUTION		
17	Ser \rightarrow Ala	1.5 \pm 0.2	14
18	Met \rightarrow Ala	1.5 \pm 0.3	5
19	Glu \rightarrow Ala	0.7 \pm 0.1	5
20	Arg \rightarrow Ala	90.5 \pm 50.0	5
21	Val \rightarrow Ala	1.7 \pm 0.2	5
22	Glu \rightarrow Ala	0.6 \pm 0.2	8
23	Trp \rightarrow Ala	>100	8
24	Leu \rightarrow Ala	67.5 \pm 14.3	5
25	Arg \rightarrow Ala	3.8 \pm 0.9	5
26	Lys \rightarrow Ala	8.3 \pm 1.2	5
27	Lys \rightarrow Ala	1.1 \pm 0.1	5
28	Leu \rightarrow Ala	9.9 \pm 1.4	5
29	Gln \rightarrow Ala	0.9 \pm 0.1	5
30	Asp \rightarrow Ala	1.1 \pm 0.2	5
31	Val \rightarrow Ala	3.8 \pm 0.6	5
Competition binding analysis for each PTH(17-31) peptide analog was performed in COS-7 cells transfected with PTH-2 receptors. IC ₅₀ is the dose of a peptide analog which inhibited by 50% the binding of ¹²⁵ I-rPTH(1-34). Each analysis was performed the number of times indicated(n).			

FIG.6



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MAP of: tether-1.seq from: 2319 to: 3698
 PTH(1-9) linked to Glu-182 of rat receptor. insert immediately
 after Tyr23 cleavage site.

```

ATGGGGGCGCCCGGATCGCACCCAGCCTGGCGCTCCTACTCTGCTGCCAGTGCTCAGC
M G A A R I A P S L A L L C C P V L S -
TCCGCcTATGCGGUUCCGAAAUCCAGCUGAUGCACggcggaggaggcGAGGTATTTGAC
S A Y A V S E I Q L M H G G G G E V F D -
CGCCTAGGCATGATCTACACCGTGGGATACTCCATGTCTCTCGCCTCCCTCACGGTGGCT
R L G M I Y T V G Y S M S L A S L T V A -
GTGCTCATCCTGGCCTATTTTAGGCGGCTGCACTGCACGCGCAACTACATCCACATGCAC
V L I L A Y F R R L H C T R N Y I H M H -
ATGTTCTGTGCTTTATGCTGCGCGCCGCGAGCATCTTCGTGAAGGACGCTGTGCTCTAC
M F L S F M L R A A S I F V K D A V L Y -
TCTGGCTTCACGCTGGATGAGGCCGAGCGCCTCACAGAGGAAGAGTTGCACATCATCGCG
S G F T L D E A E R L T E E E L H I I A -
CAGGTGCCACCTCCGCCGGCCGCTGCCGCCGTAGGCTACGCTGGCTGCCCGCTGGCGGTG
Q V P P P P A A A A V G Y A G C R V A V -
ACCTTCTTCTCTACTTCTGGCTACCAACTACTACTGGATcCTGGTGGAGGGGCTGTAC
T F F L Y F L A T N Y Y W I L V E G L Y -
TTGCACAGCCTCATCTTCATGGCCTTTTTCTCAGAGAAGAAGTACCTGTGGGGCTTCACC
L H S L I F M A F F S E K K Y L W G F T -
ATCTTTGGCTGGGGTCTACCGGCTGTCTTCGTGGCTGTGTGGGTGCGGTGTCAGAGCAACC
I F G W G L P A V F V A V W V G V R A T -
TTGGCCAACACTGGGTGCTGGGATCTGAGCTCCGGGCACAAGAAGTGGATCATCCAGGTG
L A N T G C W D L S S G H K K W I I Q V -
CCCATCCTGGCATCTGTTGTGCTCAACTTCATCCTTTTTATCAACATCATCCGGGTGCTT
P I L A S V V L N F I L F I N I I R V L -
GCCACTAAGCTTCGGGAGACCAATGCGGGCCGGTGTGACACCAGGCAGCAGTACCGGAAG
A T K L R E T N A G R C D T R Q Q Y R K -
CTGCTCAGGTCCACGTTGGTGTCTGTGCCGCTCTTTGGTGTgCACTACACCGTCTTCATG
L L R S T L V L V P L F G V H Y T V F M -
GCCTTGCCGTACACCGAGGTCTCAGGGACATTGTGGCAGATCCAGATGCATTATGAGATG
A L P Y T E V S G T L W Q I Q M H Y E M -
CTCTTCAACTCCTTCCAGGGATTTTTTGTGGCATCATATACTGTTTCTGCAATGGTGAG
L F N S F Q G F F V A I I Y C F C N G E -
GTGCAGGCAGAGATTAGGAAGTCATGGAGCCGCTGGACACTGGCGTTGGACTTCAAGCGC
V Q A E I R K S W S R W T L A L D F K R -
AAAGCACGAAGTGGGAGTAGCAGCTACAGCTATGGCCCAATGGTGTCTCACACGAGTGTG
K A R S G S S S Y S Y G P M V S H T S V -
ACCAATGTGGGCCCCCGTGCAGGACTCAGCCTCCCCCTCAGCCCCGCTGCCTCCTGCC
T N V G P R A G L S L P L S P R L P P A -
ACTACCAATGGCCACTCCCAGCTGCCTGGCCATGCCAAGCCAGGGGCTCCAGCCACTGAG
T T N G H S Q L P G H A K P G A P A T E -
ACTGAAACCCTACCAGTCACTATGGCGGTTCCCAAGGACGATGGATTCTTAACGGCTCC
T E T L P V T M A V P K D D G F L N G S -
TGCTCAGGCCTGGATGAGGAGGCCTCCGGGTCTGCGCGGCCGCTCCATTGTTGCAGGAA
C S G L D E E A S G S A R P P P L L Q E -
GGATGGGAAACAGTCATGTGA (SEQ ID NO:36)
G W E T V M * (SEQ ID NO:37)

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FIG.7

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rHA-WT

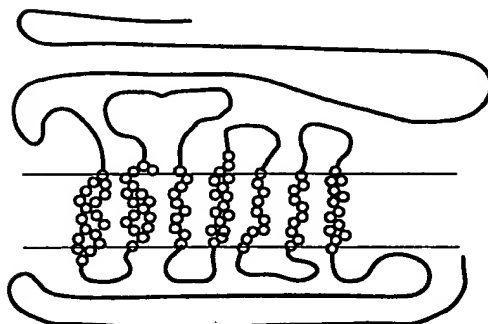


FIG.8A-1

Del-NT

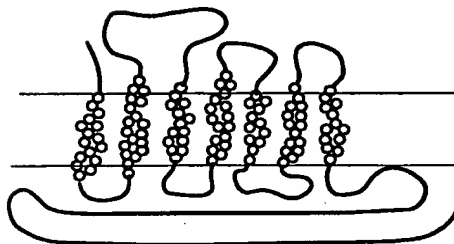


FIG.8A-2

Tether-1

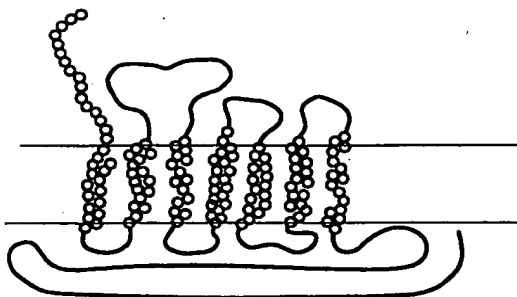


FIG.8A-3

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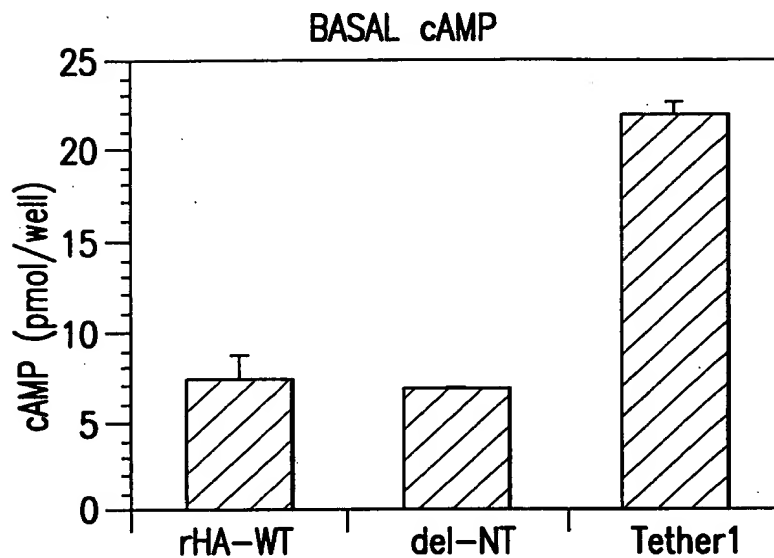
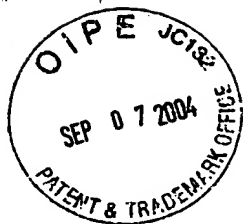


FIG.8B-1

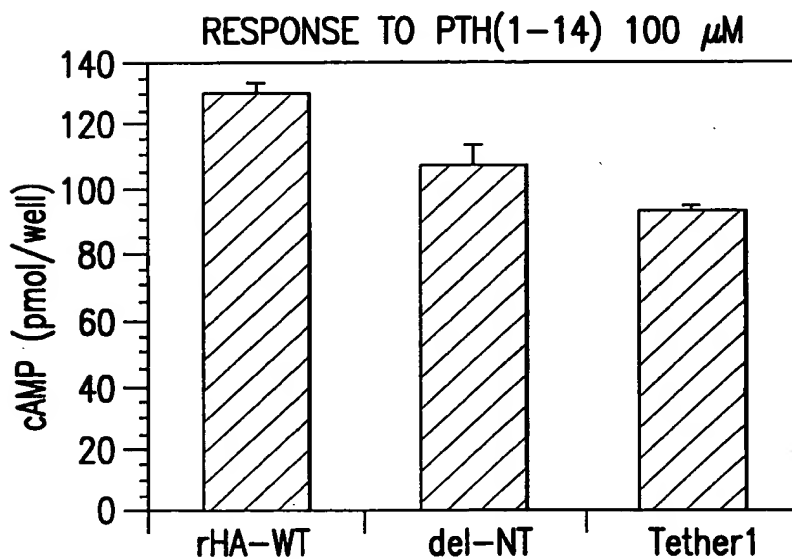


FIG.8B-2



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MAP of: tether-1C.seq check: 6795 from: 2319 to: 3326
 Stop codon at 481 added to Tether-1

```

a  ATGGGGGCGCCCGGATCGCACCCAGCCTGGCGCTCCTACTCTGCTGCCCAGTGCTCAGC
   M G A A R I A P S L A L L L C C P V L S -
a  TCCGCcTATGCGGUUCCGAAUCCAGCUGAUGCACggcgaggaggcGAGGTATTTGAC
   S A Y A V S E I Q L M H G G G G E V F D -
a  CGCCTAGGCATGATCTACACCGTGGGATACTCCATGTCTCTCGCCTCCCTCACGGTGGCT
   R L G M I Y T V G Y S M S L A S L T V A -
a  GTGCTCATCCTGGCCTATTTTAGGCGGCTGCACTGCACGCGCAACTACATCCACATGCAC
   V L I L A Y F R R L H C T R N Y I H M H -
a  ATGTTCTGTGCTTTATGCTGCGCGCCGCGAGCATCTTCGTGAAGGACGCTGTGCTCTAC
   M F L S F M L R A A S I F V K D A V L Y -
a  TCTGGCTTCACGCTGGATGAGGCCGAGCGCTCACAGAGGAAGAGTTGCACATCATCGCG
   S G F T L D E A E R L T E E E L H I I A -
a  CAGGTGCCACCTCCGCCGGCCGCTGCCGCCGTAGGCTACGCTGGCTGCCGCGTGGCGGTG
   Q V P P P P A A A A V G Y A G C R V A V -
a  ACCTTCTCCTCTACTTCCTGGCTACCACTACTACTGGATcCTGGTGGAGGGGCTGTAC
   T F F L Y F L A T N Y Y W I L V E G L Y -
a  TTGCACAGCCTCATCTTCATGGCCTTTTTCTCAGAGAAGAAGTACCTGTGGGGCTTCACC
   L H S L I F M A F F S E K K Y L W G F T -
a  ATCTTTGGCTGGGGTCTACCGGCTGTCTTCGTGGCTGTGTGGGTCGGTGTGACAGCAACC
   I F G W G L P A V F V A V W V G V R A T -
a  TTGGCCAACACTGGGTGCTGGGATCTGAGCTCCGGGCACAAGAAGTGGATCATCCAGGTG
   L A N T G C W D L S S G H K K W I I Q V -
a  CCCATCCTGGCATCTGTTGTGCTCAACTTCATCCTTTTTATCAACATCATCCGGGTGCTT
   P I L A S V V L N F I L F I N I I R V L -
a  GCCACTAAGCTTCGGGAGACCAATGCGGGCCGGTGTGACACCAGGCAGCAGTACCGGAAG
   A T K L R E T N A G R C D T R Q Q Y R K -
a  CTGCTCAGGTCCACGTTGGTGTCTGCGCGCTCTTTGGTGTgCACTACACCGTCTTCATG
   L L R S T L V L V P L F G V H Y T V F M -
a  GCCTTGCCGTACACCGAGGTCTCAGGGACATTGTGGCAGATCCAGATGCATTATGAGATG
   A L P Y T E V S G T L W Q I Q M H Y E M -
a  CTCTTCAACTCCTTCAGGGATTTTTTGTGGCATCATATACTGTTTCTGCAATGGTGAG
   L F N S F Q G F F V A I I Y C F C N G E -
a  GTGCAGGCAGAGATTAGGAAGTCATGGAGCCGCTGGACACTGGCGTaG (SEQ ID NO:38)
   V Q A E I R K S W S R W T L A * - (SEQ ID NO:39)

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FIG.9

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MAP of rdel(Nt/Ct)

```

a ATGGGGGCGCCCGGATCGCACCCAGCCTGGCGCTCCTACTCTGCTGCCAGTGCTCAGC
  M G A A R I A P S L A L L L C C P V L S -
a TCCGCATATGCGCTGGAGGTATTTGACCGCCTAGGCATGATCTACACCGTGGGATACTCC
  S A Y A L E V F D R L G M I Y T V G Y S -
a ATGTCTCTCGCCTCCCTCACGGTGGCTGTGCTCATCCTGGCCTATTTTAGGCGGCTGCAC
  M S L A S L T V A V L I L A Y F R R L H -
a TGCACGCGCAACTACATCCACATGCACATGTTCTGTGCTTTATGCTGCGCGCCGCGAGC
  C T R N Y I H M H M F L S F M L R A A S -
a ATCTTCGTGAAGGACGCTGTGCTCTACTCTGGCTTCACGCTGGATGAGGCCGAGCGCCTC
  I F V K D A V L Y S G F T L D E A E R L -
a ACAGAGGAAGAGTTGCACATCATCGCGCAGGTGCCACCTCCGCCGGCCGCTGCCGCCGTA
  T E E E L H I I A Q V P P P P A A A A V -
a GGCTACGCTGGCTGCCGCTGGCGGTGACCTTCTTCTCTACTTCTGGCTACCAACTAC
  G Y A G C R V A V T F F L Y F L A T N Y -
a TACTGGATcCTGGTGGAGGGGCTGTACTTGCACAGCCTCATCTTCATGGCCTTTTTCTCA
  Y W I L V E G L Y L H S L I F M A F F S -
a GAGAAGAAGTACCTGTGGGGCTTCACCATCTTTGGCTGGGGTCTACCGGCTGTCTTCGTG
  E K K Y L W G F T I F G W G L P A V F V -
a GCTGTGTGGGTGCGTGTGAGAGCAACCTTGGCCAACACTGGGTGCTGGGATCTGAGCTCC
  A V W V G V R A T L A N T G C W D L S S -
a GGGACAAGAAGTGGATCATCCAGGTGCCATCCTGGCATCTGTTGTGCTCAACTTCATC
  G H K K W I I Q V P I L A S V V L N F I -
a CTTTTTATCAACATCATCCGGGTGCTTGCCACTAAGCTTCGGGAGACCAATGCGGGCCGG
  L F I N I I R V L A T K L R E T N A G R -
a TGTGACACCAGGCAGCAGTACCGGAAGCTGCTCAGGTCCACGTTGGTGTCTGCGCGCTC
  C D T R Q Q Y R K L L R S T L V L V P L -
a TTTGGTGTgCACTACACCGTCTTCATGGCCTTGCCGTACACCGAGGTCTCAGGGACATTG
  F G V H Y T V F M A L P Y T E V S G T L -
a TGGCAGATCCAGATGCATTATGAGATGCTCTTCAACTCCTTCCAGGGATTTTTGTTGCC
  W Q I Q M H Y E M L F N S F Q G F F V A -
a ATCATATACTGTTTCTGCAATGGTGAGGTGCAGGCAGAGATTAGGAAGTCATGGAGCCGC
  I I Y C F C N G E V Q A E I R K S W S R -
a TGGACACTGGCGTaG (SEQ ID NO:40)
  W T L A * (SEQ ID NO:41)

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FIG.10

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Oligo is designed to join PTH(1-9) sequence to core of receptor using a Gly linker.
 Test for constitutive activation.
 Insert immediately after predicted signal peptidase cleavage site @Tyr23, use Ala24 as Ala1 of PTH.
 Join to Glu-182, = boundary of exonG/M1.

underline = flanking homology

(1-40)

ATGGGGGCGCCCGGATCGCACCCAGCCTGGCGCTCCTACTCTGCTGCCCAGTGCCTCAGC
M G A A R I A P S L A L L L C C P V L S

NDEFI ::

TCCGCATATCGCCTGGTGGATCGGACGATGCTTTACCAAGAGGAACAGATTTTCCTG (SEQ ID NO:46)
 S A Y A L V D A D D V F T K E E Q I F L - (SEQ ID NO:53)

(161-200)

553 A C C G G A C G T G G G C C A A C T A C A G C G A G T G C C T C A A G T T C A T G A C C A A T G A G A C C G G G A A 612
N R T W A N Y S E C L K F M T N E T R E .

CGGGAGGTATTTGACCGCCTAGGCATGAICTACACCGTGGGATACTCCATGTCTCTCGCC
613 - + - + - + - + - + - + - + - + - + - + - + - + - + - + - + - + - + -
R E V F D R L G M I Y T V G Y S M S L A -

(SEQ ID NO:47) (SEQ ID NO:54)

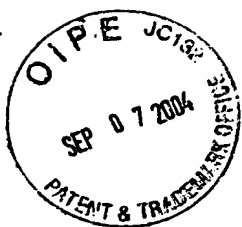
FIG. 1A

Backtranslate PTH(1-9)Gly4.:

GCUGUUUCCGAAUCCAGCUGAUGCAGCGCGAGGAGGC

Insert PTH(2-9).Gly4 between A24 and E182, use about 30 nts for flanking homology
5' flank Hom = 33 nt, 3' flank Hom = 30 nt, total = 99 nts.

FIG. 11B



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Molecules

Oligo: Sequence ID#: E16631A1

CTCTGCTGCCCAGTGCTCAGCTCCGCcTATGCGGTTTCCGAAATCCAGCTGATGCACggcgaggaggc
GAGGTATTTGACCGCCTAGGCATGATCTAC (SEQ ID NO:49)

GCG check:

MAP of: Y23E182G4PTH1-9.seq from: 1 to: 99

DNA sequence for making Tethered PTH ligand/receptor

Receptor = rat PTH1 Rec

Insert PTH(1-9)-Gly4 sequence between Tyr23 and Glu182.

Use Ala24 as codon 1 of PTH, flanking homology of ~30 nts

Takes out NDEI site at Ala 22 GCA->GCC

With 1 enzymes: NDEI

FIG.11C

a CTCTGCTGCCCAGTGCTCAGCTCCGCcTATGCGGTTTCCGAAATCCAGCTGATGCACggc
L C C P V L S S A Y A V S E I Q L M H G -

a ggaggaggcGAGGTATTTGACCGCCTAGGCATGATCTAC (SEQ ID NO:50)
G G G E V F D R L G M I Y - (SEQ ID NO:55)

Helix II: ~/rec-dna>more tether-1 map from: 2350 to:2650

Tethered PTH (1-9) to core receptor.

PTH (1-9) linked to Glu-182 of rat receptor.

Insert immediately after Tyr23 cleavage site.

Oligo tether-1 spans 2358 to 2891

takes out NDEI at 2390 Ala-22.

FIG.11D

NdeI CA'TA_TG Cuts at: 1790 1790

Size: 5769

PSORT of : rDelE1-G.seq from: 1 to:5736

*To be used as template SS DNA for Tether-1 OM.

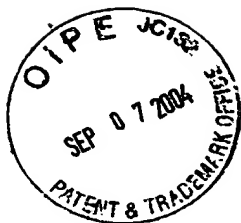
Th.1 enzymes: NDEI

NdeI CA'TA_TG

Cuts at: 1790 2384 1790

Size: 594 5142

FIG.11E



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(1-40)
Oligo Mini-HA-1:

Sequence ID#: E16853A1

CTCTGCTGCCAGTGTCTAGCTCCGCATATccctacgacgtccccgactacgccggcgaggaggcgGAGGTATTTGACCGCCTAGGCATGATCTAC (SEQ ID NO:51)

FIG.11F

MAP of: mini-HA.seq from: 1 to: 96
Oligo sequence for adding HA Epitope tag to Headless rat P1R.
Insert 9 aa tag, YPYDVPDYA, between Tyr23 and Glu182, using
Tyr 23 as codon 1 of tag, and add 4 glys for spacer. Flanking homology of ~30
nts. Takes out NDEI site at Ala 24 GCG->ccc (31-33).

With 1 enzymes: NDEI September 15, 1998 17:16 ..

CTCTGCTGCCAGTGTCTAGCTCCGCATATccctacgacgtccccgactacgccggcgga
1+.....+.....+.....+.....+.....+.....+ 60

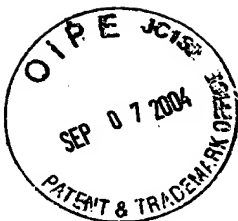
a L C C P V L S S A Y P Y D V P D Y A G G .

ggaggcGAGGTATTTGACCGCCTAGGCATGATCTAC (SEQ ID NO:52)
61+.....+.....+.....+.....+.....+.....+ 96

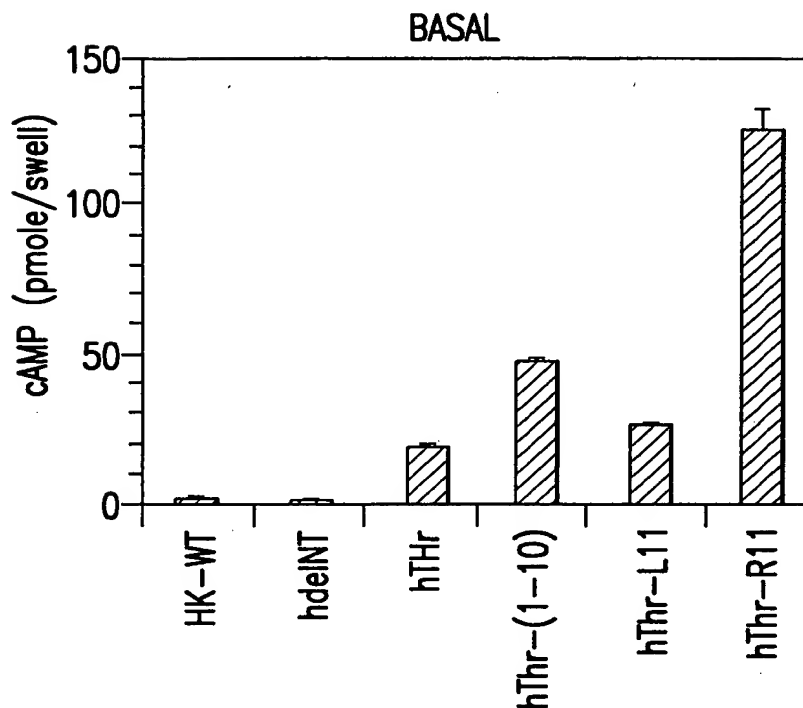
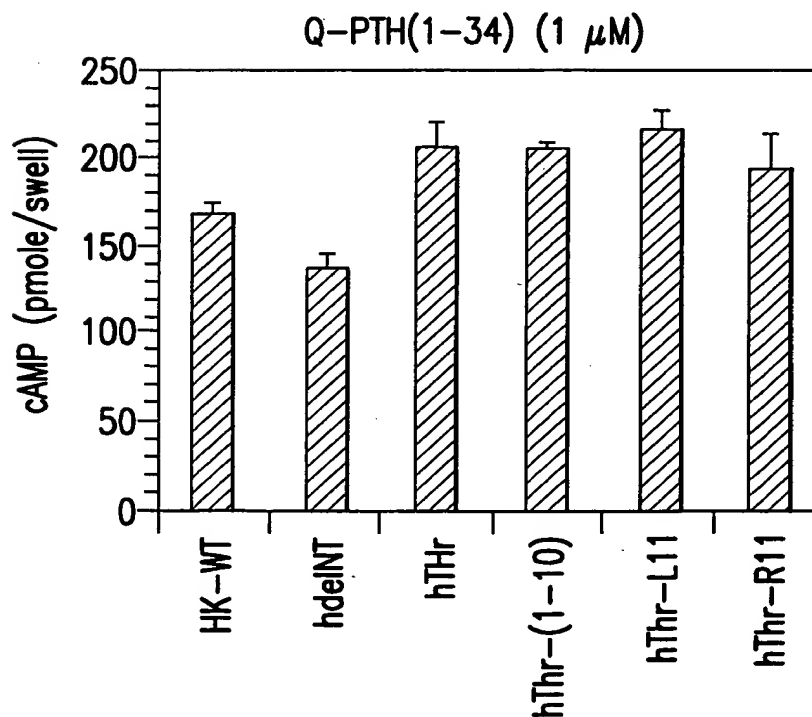
a G G E V F D R L G M I Y . (SEQ ID NO:56)

Enzymes that do not cut: NdeI

FIG.11G



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**FIG.12A****FIG.12B**



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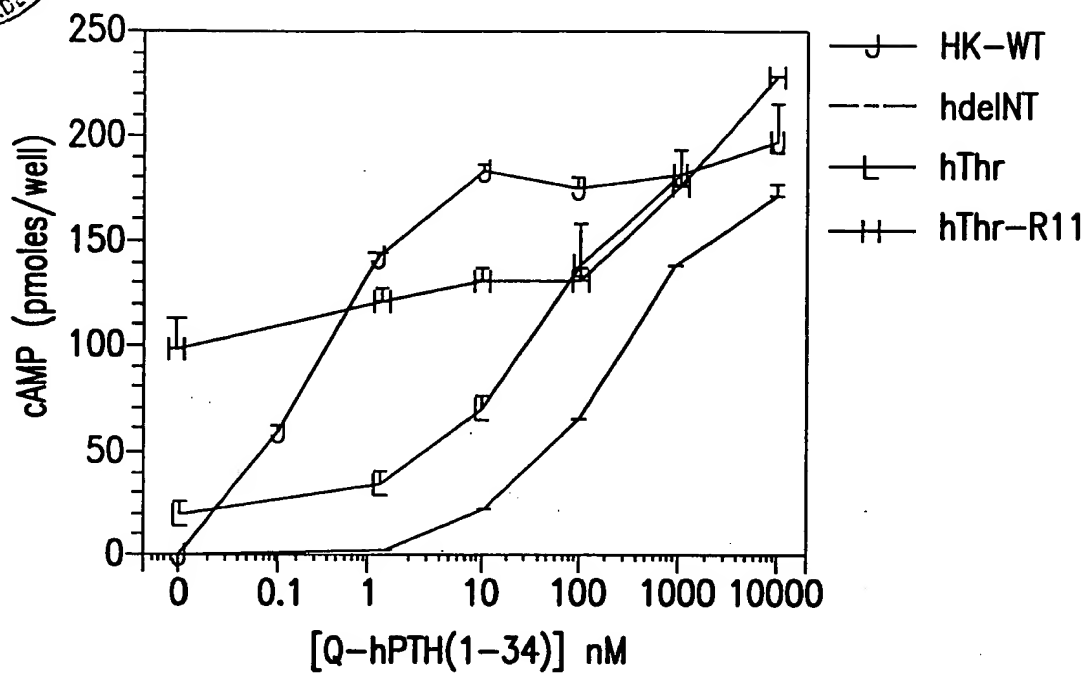


FIG.13

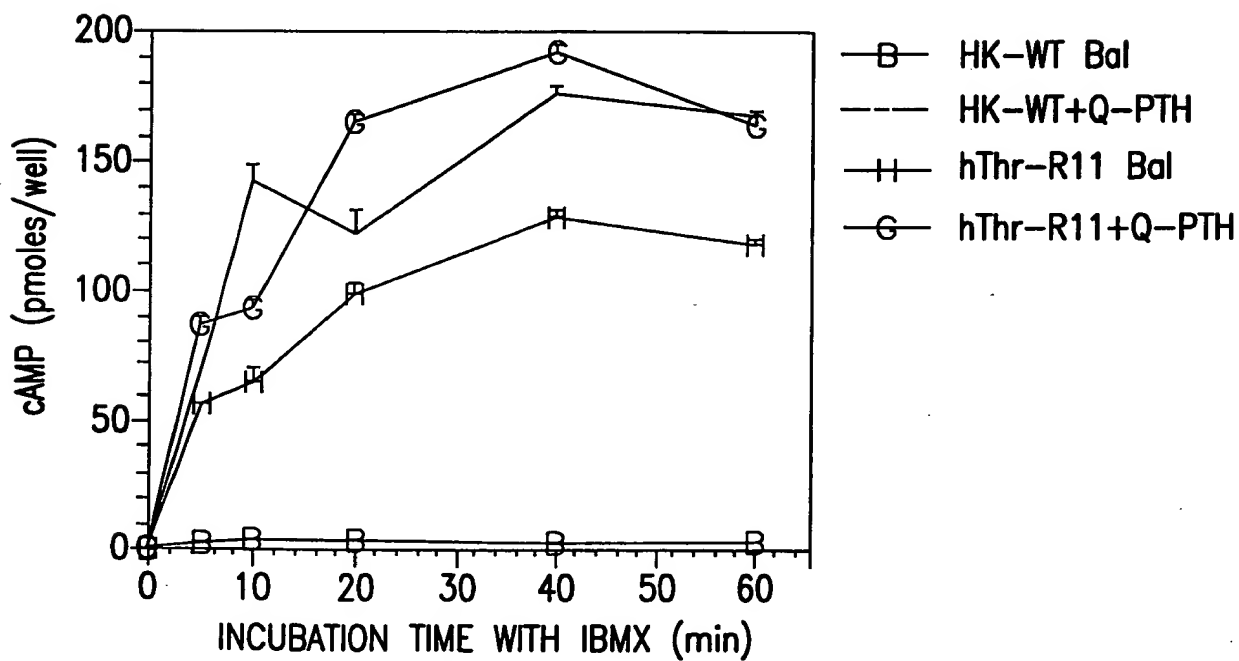


FIG.14

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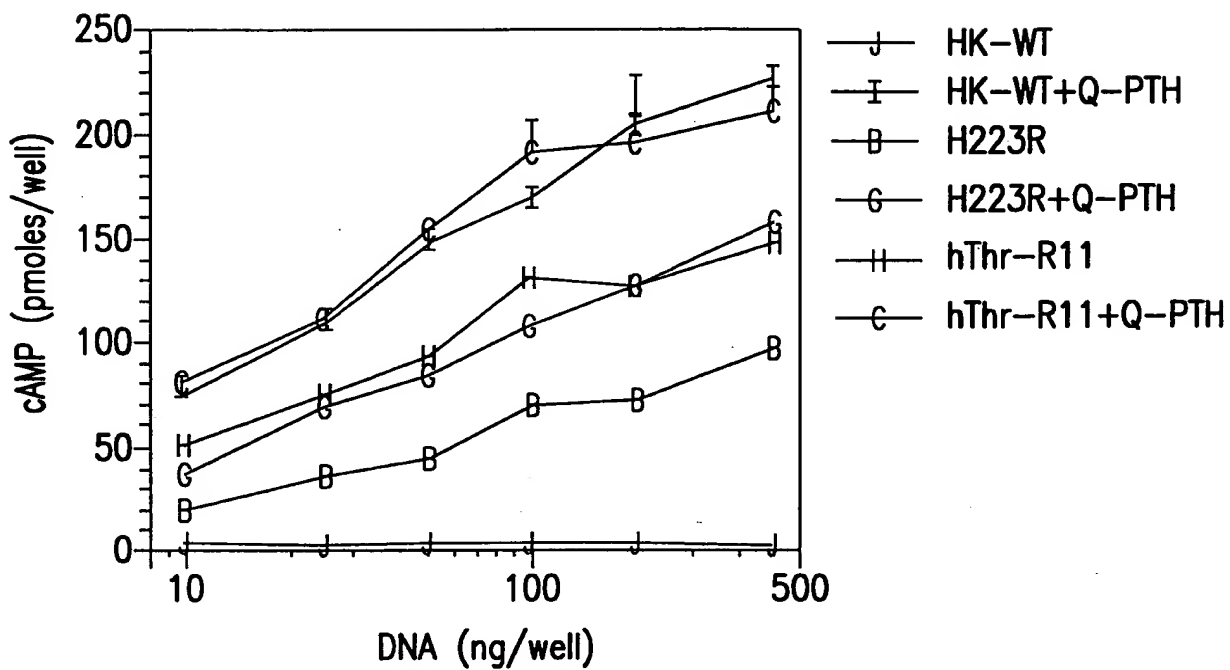


FIG.15

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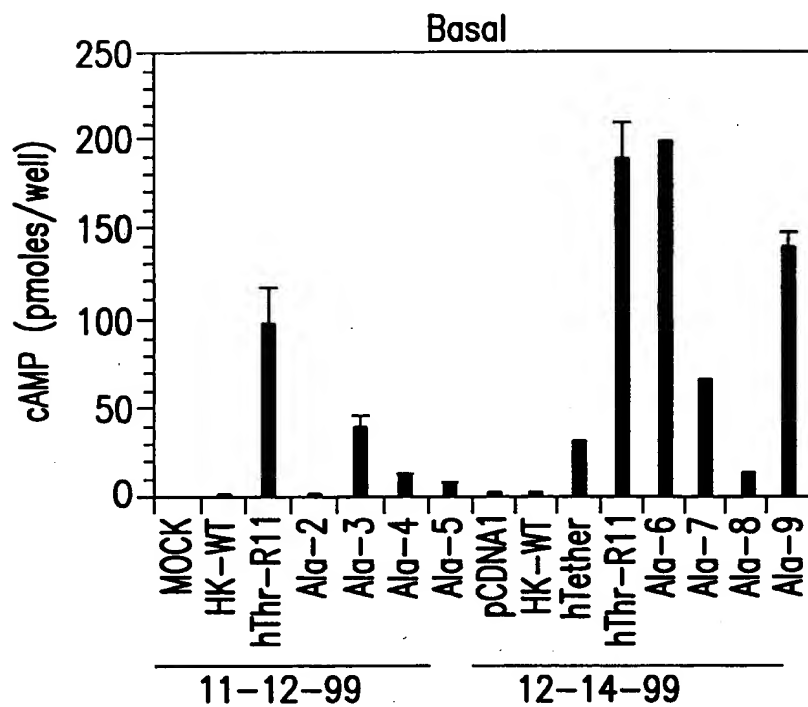


FIG.16A

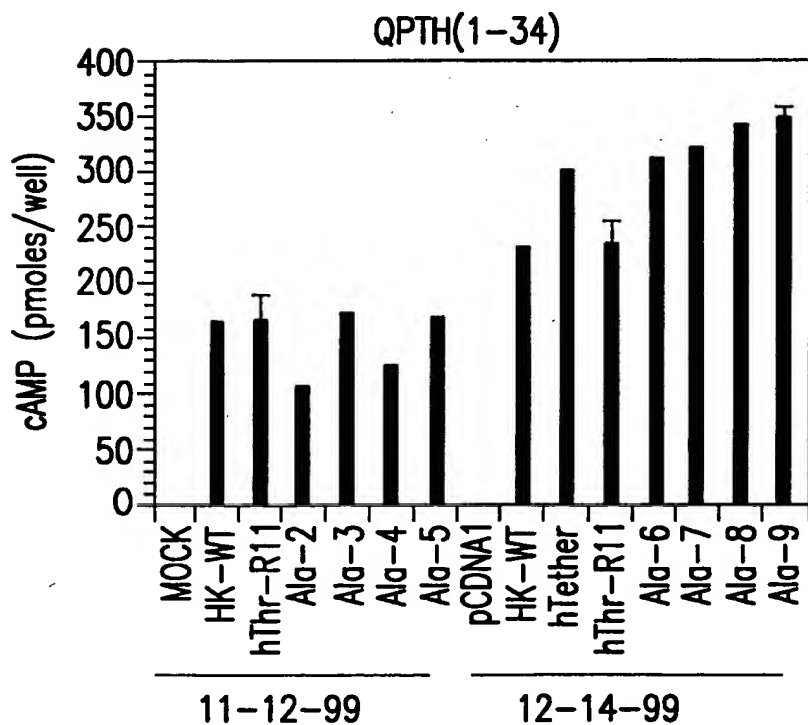
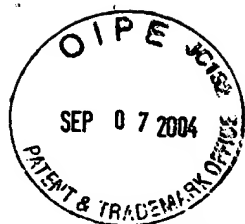


FIG.16B



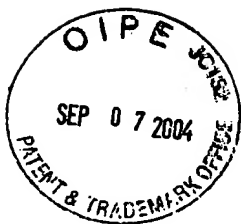
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hTether-1 From human PTH-1 receptor by replacing Ala24 to Arg181 with Ala1 to His9 of PTH, then 4-Gly linker between His9 and Glu182 by oligonucleotide mutagenesis with oligo E20986

```

      atgggGAccGCCCggatcgacccggcctggcgctcctgctctgctgccccgtgctcagc
2287  ---+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2346
a    M G T A R I A P G L A L L L C C P V L S -
      tccgcgtacgcggtttccgaaatccagctgatgcacggcggaggaggcgaggtgtttgac
2347  ---+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2406
a    S A Y A V S E I Q L M H G G G G E V F D -
      cgcctgggcatgatttacaccgtgggctactccgtgtccctggcgctccctcaccgtagct
2407  ---+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2466
a    R L G M I Y T V G Y S V S L A S L T V A -
      gtgctcatcctggcctacttttaggcggctgcactgcacgcgcaactacatccacatgcac
2467  ---+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2526
a    V L I L A Y F R R L H C T R N Y I H M H -
      ctgttcctgtccttcatgctgcgcgccgtgagcatcttcgtcaaggacgctgtgctctac
2527  ---+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2586
a    L F L S F M L R A V S I F V K D A V L Y -
      tctggcgccacgcttgatgaggctgagcgcctcaccgaggaggagctgcgcgccatcgcc
2587  ---+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2646
a    S G A T L D E A E R L T E E E L R A I A -
      caggcgccccgcgcctgccaccgccgctgccggctacgcgggctgcagggtggctgtg
2647  ---+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2706
a    Q A P P P P A T A A A G Y A G C R V A V -
      accttcttctttacttcttggccaccaactactactggattcttgggtggaggggctgtac
2707  ---+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2766
a    T F F L Y F L A T N Y Y W I L V E G L Y -
      ctgcacagcctcatcttcatggccttcttctcagagaagaagtacctgtggggcttcaca
2767  ---+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2826
a    L H S L I F M A F F S E K K Y L W G F T -
      gtcttcgggctgggggtctgcccgctgtcttcgtgggctgtgtgggtcagtgctcagagctacc
2827  ---+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2886
a    V F G W G L P A V F V A V W V S V R A T -
      ctggccaacaccgggtgctgggacttgagctccgggaacaaaaagtggatcatccagggtg
2887  ---+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 2946
a    L A N T G C W D L S S G N K K W I I Q V -
      cccatcctggcctccattgtgctcaacttcatcctcttcatcaatatcgtccgggtgctc
2947  ---+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 3006
a    P I L A S I V L N F I L F I N I V R V L -
      gccaccaagctgcgggagaccaacgccggccgggtgtgacacacggcagcagtagaccggaag
3007  ---+-----+-----+-----+-----+-----+-----+-----+-----+-----+ 3066
a    A T K L R E T N A G R C D T R Q Q Y R K -
  
```

FIG.17A



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```

ctgctcaaatccacgctgggtgctcatgcccctctttggcgtccactacattgtcttcatg
3067 ---+-----+-----+-----+-----+-----+-----+-----+----- 3126
a  L L K S T L V L M P L F G V H Y I V F M -
   gccacaccatacaccgaggtctcagggacgctctggcaagtccagatgcactatgagatg
3127 ---+-----+-----+-----+-----+-----+-----+-----+----- 3186
a  A T P Y T E V S G T L W Q V Q M H Y E M -
   ctcttcaactccttccagggattttttgtcgcaatcatatactgtttctgcaatggcgag
3187 ---+-----+-----+-----+-----+-----+-----+-----+----- 3246
a  L F N S F Q G F F V A I I Y C F C N G E -
   gtacaagctgagatcaagaaatcttggagccgctggacactggcactggacttcaagcga
3247 ---+-----+-----+-----+-----+-----+-----+-----+----- 3306
a  V Q A E I K K S W S R W T L A L D F K R -
   aaggcagcagcgggagcagcagctatagctacggcccatggtgtcccacacaagtgtg
3307 ---+-----+-----+-----+-----+-----+-----+-----+----- 3366
a  K A R S G S S S Y S Y G P M V S H T S V -
   accaatgtcggcccccggtgtgggactcggcctgcccctcagccccgcctactgccact
3367 ---+-----+-----+-----+-----+-----+-----+-----+----- 3426
a  T N V G P R V G L G L P L S P R L L P T -
   gccaccaccaacggccaccctcagctgcctggccatgccaagccagggaccccagccctg
3427 ---+-----+-----+-----+-----+-----+-----+-----+----- 3486
a  A T T N G H P Q L P G H A K P G T P A L -
   gagaccctcgagaccacaccacctgccatggctgctcccaaggacgatgggttcctcaac
3487 ---+-----+-----+-----+-----+-----+-----+-----+----- 3546
a  E T L E T T P P A M A A P K D D G F L N -
   ggctcctgctcaggcctggacgaggaggcctctgggcctgagcggccacctgccctgcta
3547 ---+-----+-----+-----+-----+-----+-----+-----+----- 3606
a  G S C S G L D E E A S G P E R P P A L L -
   caggaagagtgggagacagtcatgtgaccaggcgtgggggct (SEQ ID NO:61)
3607 ---+-----+-----+-----+-----+-----+-----+-----+----- 3649
a  Q E E W E T V M * (SEQ ID NO:62)

```

FIG.17B

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hde1NT

human PTH-1 res. deleted for 24-181; Joins Tyr23 to Glu182.

February 25, 1999 13:38

a	2260	TGGATCCCGCGCCCTAGCGGTGGCGGatgggGAccGccggatgcaccgcgcctggcg	2319
		ctcctgctctgctgccccgtgctcagctcgcAtaIgaggtgtttgaccgcctgggcatg	
a	2320	L L L C C P V L S S A Y E V F D R L G M	2379
		atttacaccgtgggtactcctgctccctggcgtccctcaccgtagctgtgctcctg	
a	2380	I Y T V G Y S V S L A S L T V A V L I L	2439
		gcctactttagggcggtgactgcacgcgcaactacatccacatgcacctgttctgtcc	
a	2440	A Y F R R L H C T R N Y I H M H L F L S	2499
		ttcatgctgcgcgcgtgagcatcttcgtcaaggacgctgtgcttactctggcgccacg	
a	2500	F M L R A V S I F V K D A V L Y S G A T	2559
		cttgatgaggtgagcgctcaccgaggagagctgcgcgcacatgcgccagcgcccccg	
a	2560	L D E A E R L T E E L R A I A Q A P P	2619
		ccgcctgccaccgcgtgcggctacgcggctgagggctgagggctgtgaccttcttcctt	
a	2620	P P A T A A A G Y A G C R V A V T F F L	2679
		tacttctggccaccaactactactggttctgtgagggtgtacctgcacagcctc	
a	2680	Y F L A T N Y Y W I L V E G L Y L H S L	2739
		atcttcatggccttctctcagagaagaaglacctgtggggttccacagtcttcggctgg	
a	2740	I F M A F S E K K Y L W G F T V F G W	2799
		ggtctgccgcgtgtctctgtggtgtggtcagtgtagctaccctggccaacacc	
a	2800	G L P A V F V A V W V S V R A T L A N T	2859
		gggtgctgggacttgagctccgggaacaaaagtgatcatccaggtgcccatcctggcc	
a	2860	G C W D L S S G N K K W I I Q V P I L A	2919
		tccattgtgctcaacttcattcttcatcaatatcgtcgggtgctcgccaccaagctg	
a	2920	S I V L N F I L F I N I V R V L A T K L	2979
		cgggagaccacgcggcggtgtgacacacggcagcagtagtaccggaagctgtcctaatcc	
a	2980	R E T N A G R C D T R Q Q Y R K L L K S	3039

FIG. 18A

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3040	a	acgctggtgctcatgccccctcttttggcgctccactacattgtcttcatggccacaccatac	3099
		T L V L M P L F G V H Y I V F M A T P Y	
3100	a	accgaggtctcagggaagctctggcaagtcagatgcactatgagatgctcttcaactcc	3159
		T E V S G T L W Q V Q M H Y E M L F N S	
3160	a	ttccagggatttttgcgaatcatactgtttctgcaatggcgaggtacaaagctgag	3219
		F Q G F F V A I I Y C F C N G E V Q A E	
3220	a	atcaagaaatcttggagccgctggacactggcactggacgttcaagcgaaggaagcagcagc	3279
		I K K S W S R W T L A L D F K R K A R S	
3280	a	gggagcagcagctatagctacggccccctgtgttccacacaaagtgtgaccaatgtcggc	3339
		G S S Y S Y G P M V S H T S V T N V G	
3340	a	ccccgtgtgggactcggcctgccccctcagccccgcctactgccccactgccaccaccaac	3399
		P R V G L G L P L S P R L L P T A T T N	
3400	a	ggccaccctcagctgcctggccatgccaaagccaggaccgccccagccctggagaccctcgag	3459
		G H P Q L P G H A K P G T P A L E T L E	
3460	a	accacaccactgccatggctgtcctccaaaggacgatgggttctcctcaacggctcctgctca	3519
		T T P P A M A A P K D D G F L N G S C S	
3520	a	ggcctggacgagggagcctctggcctgagcggccacctgcccctgctacaggaagagtgg	3579
		G L D E E A S G P E R P A L L Q E E W	
3580	a	gagacagtcatgtgaccagggcgtggggcctggacctgctgacatagtggatggacagat	3639
		E T V M *	

(SEQ ID NO:59)
 (SEQ ID NO:60)

FIG.18B

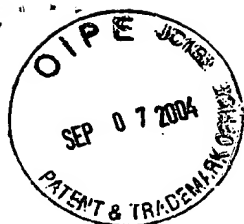


hTether-R11
Made from hTether-1 by inserting Asn10-Arg11 between His9 and first Gly of linker by oligonucleotide mutagenesis with Oligo = E27309

[illegible]

FIG. 19A

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2947	a	caggtgcccatcctggcctccattgtgtcctaacttcacatctcttcacatcaatctcgtccgg	3006
		Q V P I L A S I V L N F I L F I N I V R	
3007	a	gtgctcgccaccaagctcgaggagaccaacgccggcggtgtgacacacggcagcagtac	3066
		V L A T K L R E T N A G R C D T R Q Q Y	
3067	a	cggaaagtgtcctcaaatccacgctggtgtcctatgccctctttggctccactacattgtc	3126
		R K L L K S T L V L M P L F G V H Y I V	
3127	a	ttcatggccacaccatacacagggtctcaggagcgtctggaagtccagatgcactat	3186
		F M A T P Y T E V S G T L W Q V Q M H Y	
3187	a	gagatgctcttcaactccttcagggtatttttgcgaatcatatactgtttctgcaat	3246
		E M L F N S F Q G F F V A I I Y C F C N	
3247	a	ggcgaggtacaagctgagatcaagaatcttgagccgctggacactggcactggacttc	3306
		G E V Q A E I K K S W S R W T L A L D F	
3307	a	aagcgaaaggcacgcagcgggagcagcagctatagctacggccccatggtgtccacaca	3366
		K R K A R S G S S Y S Y G P M V S H T	
3367	a	agtgtgaccaatgtcgcccccggtgtggactcggcctgccccctcagcccccgctactg	3426
		S V T N V G P R V G L G L P L S P R L L	
3427	a	cccactgccaccaccaacggccaccctcagctgcctggccatgccaaagccaggacccca	3486
		P T A T T N G H P Q L P G H A K P G T P	
3487	a	gccctggagaccctcgagaccacaccacctgccatggctgctcccaaggacgatgggttc	3546
		A L E T L E T T P P A M A A P K D D G F	
3547	a	ctcaacggctcctgctcaggcctggacgaggagcctctggcctgagcggccacctgcc	3606
		L N G S C S G L D E E A S G P E R P A	
3607	a	ctgctacaggaagatgggagacagtcatgtgaccaggcgtggggctggacctgctga	3666
		L L Q E E W E T V M *	

(SEQ ID NO:57)

(SEQ ID NO:58)

FIG.19B